Cellular Responses to Mechanical Stress

C. C. DOS SANTOS AND A. S. SLUTSKY
Department of Medicine, St. Michael’s Hospital, and University of Toronto,
Toronto, Ontario, Canada M5B 1W8

Dos Santos, C. C. and A. S. Slutsky. Invited Review: Mechanisms of ventilator-induced lung injury: a perspective. J Appl Physiol 89: 1645–1655, 2000.—Despite advances in critical care, the mortality rate in patients with acute lung injury remains high. Furthermore, most patients who die do so from multisystem organ failure. It has been postulated that ventilator-induced lung injury plays a key role in determining the negative clinical outcome of patients exposed to mechanical ventilation. How mechanical ventilation exerts its detrimental effect is as of yet unknown, but it appears that overdistension of lung units or shear forces generated during repetitive opening and closing of atelectatic lung units exacerbates, or even initiates, significant lung injury and inflammation. The term “biotrauma” has recently been elaborated to describe the process by which stress produced by mechanical ventilation leads to the upregulation of an inflammatory response. For mechanical ventilation to exert its deleterious effect, cells are required to sense mechanical forces and activate intracellular signaling pathways able to communicate the information to its interior. This information must then be integrated in the nucleus, and an appropriate response must be generated to implement and/or modulate its response and that of neighboring cells. In this review, we present a perspective on ventilator-induced lung injury with a focus on mechanisms and clinical implications. We highlight some of the most recent findings, which we believe contribute to the generation and propagation of ventilator-induced lung injury, placing a special emphasis on their implication for future research and clinical therapies.

mechanical ventilation; mechanotransduction; biotrauma; ventilator-induced lung injury; acute respiratory distress syndrome

Despite advances in critical care, the mortality rate in patients with acute respiratory distress syndrome (ARDS) remains high at values exceeding 30%. Furthermore, most patients who die do so from multisystem organ failure (MSOF) rather than from hypoxia (38), an observation that has puzzled both clinicians and scientists interested in acute lung injury (ALI). One hypothesis that has recently been advanced to explain this observation is that mechanical ventilation per se may be responsible not only for worsening the underlying ALI but, by a number of mechanisms, may also lead to the development of a systemic inflammatory response syndrome (SIRS) (14, 58, 59) and MSOF (60, 66). The postulate is that overdistension of lung units and/or shear forces generated during repetitive opening and collapse of atelectatic regions of the lung exacerbate, or even initiate, significant lung injury and inflammation (41, 59, 60), with or without mechanical ventilation; mechanotransduction; biotrauma; ventilator-induced lung injury; acute respiratory distress syndrome
concomitant gross structural disruption. Ventilator-induced lung injury (VILI) is thus the result of a complex interplay among various mechanical forces acting on lung structures during mechanical ventilation. The weight of evidence obtained from experimental animal studies, correlative human studies, and interventional human studies addressing the detrimental effects of different ventilatory strategies quite convincingly points to the role of this entity in determining clinically significant outcomes in ventilated patients (59, 66). Moreover, it is in the wake of a recently published National Institutes of Health trial (64) that the full impact of ventilator-associated lung injury becomes evident.

The Acute Respiratory Distress Network (63) recently reported results for 861 patients with ALI and ARDS who were randomly assigned to receive ventilation with either a conventional tidal volume (12 ml/kg of predicted body weight) or a low tidal volume (6 ml/kg). The trial was stopped when an interim analysis revealed that the lower tidal volume group had a mortality that was 22% lower than the higher tidal volume group (63). Thus this trial has strikingly shown that mechanical ventilation can lead to iatrogenic consequences, similar to virtually all therapies in the critical care unit setting. Nonetheless, mechanical ventilation is clearly an indispensable tool for patients who require ventilatory support; however, if we are to succeed in eliminating its iatrogenic consequences, we must understand the biomolecular and cellular effects of exposing the lung to the forces generated by mechanical ventilation. Moreover, if correct, this new conceptualization of VILI could lead to a paradigm shift in which therapies to prevent VILI are not solely based on changes in ventilatory strategies to limit mechanical injury but are also aimed at constraining and/or modulating the inflammatory response.

Exactly how mechanical ventilation induces its deleterious effects is as of yet unclear. Studies in vitro and in vivo have found that both the pattern and the degree of stretch are important in determining cellular response (59, 66). In fact, lung stretch is known to be an important factor in lung growth and development (31), as well as surfactant production (51, 79). The postulate is therefore that, by alternating both pattern and magnitude of stretch, mechanical ventilation may lead to alterations in gene expression and/or cellular metabolism. Recently, a new mechanism of injury, termed “biotrauma,” has been elaborated in which the mechanical stress produced by mechanical ventilation leads to upregulation of an inflammatory response (66), as evidenced by neutrophil infiltration in the lungs and increased bronchoalveolar lavage (BAL) levels of host inflammatory mediators (44, 60, 70). It is thought that, as compartmentalization of the local pulmonary response is lost, systemic release of inflammatory mediators promotes the massive inflammatory response that underlies MSOF (59, 60, 66). This is rapidly followed by the generation of an equally dramatic compensatory anti-inflammatory reaction that is designed to downregulate and attenuate the proinflammatory response (60, 66). Loss of appropriate immune modulation, or persistence of inflammatory injury, appears to be involved in the inability of organisms to bring about resolution of the proinflammatory response, which ultimately leads to death (10, 12, 60).

Clinical evidence in support of this model for the development of MSOF in ventilated ARDS patients became available when Ranieri et al. (49) demonstrated that the concentrations of proinflammatory cytokines in both BAL fluid and plasma could indeed be decreased in patients ventilated with a lung-protective strategy. Patients in the lung-protective strategy group had reductions in polymorphonuclear (PMN) cells and proinflammatory and anti-inflammatory cytokines in both their BAL and serum concentrations (49). In a follow-up to this study, the authors demonstrated that changes in plasma concentrations of some of these mediators correlated with changes in the development of organ dysfunction (19).

In addition, ventilatory models that allow end-expiratory collapse can induce bacterial translocation from the lung to the systemic circulation when very high tidal volumes are used (42, 74); even strategies that use relatively normal tidal volumes can induce endotoxin translocation from the lung to the systemic circulation (40). Evidence that important inflammatory mediators can escape the confines of the lung provides important clues to the mechanisms that potentially lead to the development of MSOF in VILI. Moreover, in a recently presented experimental study (55), the use of protective lung strategies with low tidal volume delayed bacteremia and consequent distal injury.

In this review, we shall attempt to highlight some of the most recent and pertinent findings that have contributed to our understanding of the mechanisms responsible for the initiation and progression of VILI, its role in the propagation of an inflammatory response, and the potential generation of MSOF. This review is designed to be a perspective on VILI with a special focus on the potential future research directions and treatment implications of current findings.

**PUTATIVE MECHANISMS FOR SENSING MECHANICAL FORCES**

Mechanotransduction is the conversion of mechanical stimuli, such as cell deformation, into biochemical and biomolecular alteration. Mechanical forces important to pulmonary structure and function are produced by gradients in gravity, motion, osmotic forces, and interactions between cells and/or cell matrix (for a review on the effects of mechanical forces on lung function, see Ref. 80). How mechanical forces can be sensed by cells (mechanosensors) and converted into biochemical signals for intracellular signal transduction is still unknown. Moreover, because of the complexity of the pulmonary organ structure, the variety of cell types, and the variety of physical forces to which cells are exposed, possible mechanisms of mechanical stimulation and the potential cellular responses induced may vary widely (for a review on mechanical
force-induced signal transduction in the lung, see Ref. 32). In the following sections, we will discuss selected mechanisms by which it has been proposed that cellular deformation is converted into cellular phenotype and how these mechanisms may be responsible for the generation of VILI.

**Stretch-sensitive channels (Fig. 1A).** Stretch-activated ion channels are known to play important roles in both pulmonary physiology and development (32). However, what specific role they play in VILI is yet to be determined. Recent work however has highlighted the potential involvement of at least cation channels in mediating the inflammatory response generated in the lung after a mechanical stress. Loss of barrier function, inability of cells to preserve vascular permeability, is one of the hallmarks of ARDS and VILI. Parker et al. (45) studied the initial signaling events that caused the increase in vascular permeability after high airway pressure injury. They found that the increases in microvascular permeability were abolished by gadolinium (blocks stretch-activated nonselective cation channels) and hence concluded that stretch-activated cation channels may initiate the increase in permeability induced by mechanical ventilation through increases in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_i$) (45).

Recently, Waters et al. (78) postulated that a stretch-activated cation channel may also regulate Na$^+$-K$^+$-ATPase activity, a factor that may play an important role in lung edema clearance. In epithelial cells, Na$^+$ moves across its concentration gradient through Na$^+$ channels on the luminal side of the cell. Na$^+$ transport out of cells is carried out by the Na$^+$-K$^+$-ATPase pump on the basolateral aspect of the cell. Waters et al. demonstrated that, after 30 and 60 min of cyclical stretch (30 cycles/min), Na$^+$-K$^+$-ATPase activity was significantly increased in murine lung epithelial cells. When cells were treated with amiloride (blocks amiloride-sensitive Na$^+$ entry into cells) or with gadolinium, there was no stimulation of Na$^+$-K$^+$-ATPase. Interestingly, changes in Na$^+$-K$^+$-ATPase activity were paralleled by increased Na$^+$-K$^+$-ATPase protein in the basolateral membrane of epithelial cells, suggesting that increased recruitment of these channel subunits from intracellular pools to the basolateral membrane may be induced by cyclic stretch (78). Moreover, the rat epithelial amiloride-sensitive sodium channel (reNaC) has been shown to be a mammalian homologue of mechanosensitive channels in *Caenorhabditis elegans* (23, 24). These channels may represent clinically significant targets for site-directed mutagenesis in future treatment strategies for VILI.

**Plasma membrane integrity (Fig. 1B).** The role of structural damage to cells as the inciting inflammatory event is currently being investigated. To date, there is no substantial evidence suggesting that cytopathological changes mediate the inflammatory response characteristic of VILI. Nevertheless, we believe that maintenance of plasma membrane integrity undoubtedly plays an integral role in important intracellular as well as extracellular signaling pathways relevant to VILI. Here, we present two studies that illustrate the potential significance of plasma membrane stress failure as an inciting mechanism by which cells sense and respond to mechanical injury.

Hinman et al. (22) demonstrated that, when alveolar type II cells (ATII) were injured, elevations in free [Ca$^{2+}]_i$ began at the edge of the wound and propagated outward as a wave for at least 300 μm. The [Ca$^{2+}]_i$ wave was due both to influx of extracellular Ca$^{2+}$ and to release of intracellular Ca$^{2+}$ stores. [Ca$^{2+}]_i$ elevations propagated over a break in ATII monolayers and caused elevations in [Ca$^{2+}]_i$ in uninjured cells (22). Previous studies demonstrated that changes in Ca$^{2+}$ homeostasis can affect not only the signaling pathways in which Ca$^{2+}$ itself serves as a signaling component but also the signaling system turned on by other sepsis-induced agonists that may not be dependent on Ca$^{2+}$ signaling. In fact, the increase in apparent basal [Ca$^{2+}]_i$ in sepsis can hypersensitize phosphokinase C (PKC) activation, an important protein in signal transduction pathways (for a review on alterations in Ca$^{2+}$ signaling and cellular response in septic injury, see Ref. 56). We speculate that alteration of [Ca$^{2+}]_i$, may also contribute to mechanical force-initiated signal transduction and cellular injury in VILI.

Grembowicz et al. (20) recently demonstrated that plasma membrane disruption (PMD) induced an increase in Fos protein synthesis. This increase was shown to be highly specific and occurred primarily in cells lining the membrane disruption tracts (where breaks in the cell membrane occurred) (20). The expression of the *fos* gene can be induced by Ca$^{2+}$-5, and c-fos is known to contain a Ca$^{2+}$ response element in its cis-regulatory region (18). The importance of c-fos resides not only in the fact that it is an early response gene with a stretch-responsive promoter element but also in its central role in mediating gene transcription; c-fos mRNA has been shown to be increased by injurious ventilatory strategies in an isolated rat lung model (67). Whether injurious mechanical ventilation induces changes in Fos protein as well as PMD needs to be elucidated. Moreover, PMD has also been shown to induce translocation of nuclear factor-κB (NF-κB) into the nucleus of mechanically injured human endothelial smooth muscle cells (20). As discussed below, NF-κB translocation and activation are essential steps for expression of several proinflammatory cytokines and chemokines. Furthermore, the role of other cytopathological changes caused by structural damage, such as blebbing, gap formation, cell lysis, and debris accumulation, is as yet unknown.

**Direct conformational change in membrane-associated molecules (Fig. 1C).** It has recently become evident that the structural organization and interconnectedness of the cytoskeleton provides a physical basis for translating mechanical forces into biochemical response, as well as a mechanism for integrating these signals with those generated by growth factors and molecular components of the extracellular matrix (for an integrative review, see Ref. 28). Recent experimental studies suggest that cell surface adhesion proteins (e.g., integrins, cell-cell adhesion molecules), the cy-
toskeleton, and associated nuclear scaffolds function as a structurally unified system that provides the architectural infrastructure that enables cells to respond to mechanical forces transmitted over its surface. This appears to be mediated through specialized anchoring complexes or focal adhesion complexes where mechanical coupling between components of the cell’s architecture and molecules that transduce signals into the cell seems to occur.

Evidence suggesting that cell matrix and adhesion molecules may be important in mediating the inflammatory response characteristic of VILI comes from a number of studies. Deformation of the airway wall during bronchoconstriction has been postulated to be transmitted to the airway epithelium via cell-cell and cell-matrix attachments. Therefore, Tschumperlin et al. (68) subjected pulmonary epithelial cells to magnetic twisting cytometry (MTC), a technique in which ferromagnetic beads are coated with a ligand and bound to the cell surface through integrins (a family of transmembrane heterodimers that form part of the cellular cytoskeleton). The beads are then magnetized, and subsequent application of an external magnetic field results in an applied torque (or twisting stress). Bead rotation is opposed by reaction forces generated within the cytoskeleton to which the bead is bound. MTC measures the applied twisting stress and the resulting angular rotation of the magnetic bead and expresses the ratio of cell stiffness. This stiffness is a measure of the ability of the cell to resist distortion to shape (77). Tschumperlin et al. examined gene expression as a result of MTC. Early growth response protein (Egr-1), tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β) transcription increased in response to magnetic twisting applied to beads coated with collagen and not to control beads (68). In this case, the collagen functions as a ligand protein for transmembrane cytoskeletal proteins. The epithelial response peaked at much different field strengths, corresponding to differing amounts of mechanical stress (68). These findings indicate that both the magnitude and specific cell-matrix connections are important determinants of the airway epithelial response to mechanical force. Because actin and myosin constitute an important part of the cytoskeleton, Hubmayr et al. (25) hypothesized that changes in actin-myosin interactions might mediate changes in cell stiffness. They demonstrated that human airway smooth muscle cell stiffness increased when cells were exposed to contractile agonists, previously documented to have an effect on actin-myosin interactions. Contractile agonists utilized in this study mediated their action by increasing [Ca^{2+}], thereby activating the contractile apparatus. Moreover, they cultured cells in the presence of inflammatory mediators (bradykinin and histamine) and demonstrated an increase in cell stiffness. Subsequent treatment of these cells with different agents such as a bronchodilator agonist and prostaglandin E_{2} demonstrated a dose-dependent decrease in cell stiffness.

Bhullar et al. (8) showed that preincubation of endothelial cells with a monoclonal anti-integrin antibody attenuates shear stress induction of an inhibitor of NF-κB kinase, IkB kinase (IKK). Inhibition of tyrosine kinase caused a similar downregulatory effect, suggesting that an integrin-mediated signaling pathway regulates NF-κB through IKKs, at least in endothelial cells (8). If this is true in pulmonary cells involved in the ALI response, it might have important implications for potential targeting in the context of novel molecular therapy.

**GENERATION OF INTRACELLULAR SIGNALING PATHWAYS RELATED TO VILI**

Transcription factors are DNA-binding proteins that regulate gene expression. In the nucleus, physical forces can exert their effects by influencing expression of immediate early response genes, some of which are transcription factors such as c-fos, c-jun, c-myc, and Egr-1. It has been demonstrated that most early response genes contain stretch-response elements in their cis-acting regulatory sequences (60, 67). Putative transcriptional factor binding sites that contain shear-stress-responsive elements have also been demonstrated in cis-acting regulatory regions of various genes, including platelet-derived growth factor-B (lung development), tissue plasminogen activator (thrombosis), intracellular adhesion molecule-1 (ICAM-1; neutrophil sequestration), and TGF-β (lung fibrosis) (32). In addition, genes that encode for nitric oxide synthase and cyclooxygenase-2 have been shown to be regulated by shear stress (50, 71) and cyclic strain (4).

Current evidence suggests that the activation and control of NF-κB plays a critical role in the generation and propagation of the cytokine response in VILI. NF-κB is known to be a key transcription factor for maximal expression of many cytokines that are involved in the pathogenesis of inflammatory diseases, such as ARDS and sepsis syndrome (for a review of NF-κB in cytokine gene regulation, see Ref. 9). NF-κB can be activated in cells by a variety of stimuli, including bacterial endotoxin, TNF-α, interleukin (IL)-1β, mitogens, and viral proteins. NF-κB itself contains a DNA “shear-stress” response element at its promoter region, and NF-κB protein binds to the IL-6, IL-8, IL-1β, and TNF-α promoter sequences (9). A number of studies in both in vitro and ex vivo whole lung preparations have shown that NF-κB is upregulated in response to stretch (30, 35, 57, 65).

In quiescent cells, NF-κB is sequestered in the cytoplasm through its interaction with the inhibitors of NF-κB (IκB). Phosphorylation of IκB after cellular activation unmasks the nuclear localization signal that allows for NF-κB transportation to the nucleus and binding to DNA regulatory sequences. Regulation of NF-κB may occur at any point of its activation and postactivation pathway and likely plays a central role in NF-κB-mediated inflammatory cascade. In ARDS for example, Moine et al. (37) showed that, despite increases in IκB levels and decreases in Bcl-3 levels...
(member of the IκB family), NF-κB remained activated (37). These results suggest that fundamental abnormalities in transcriptional mechanisms involving NF-κB are likely important in the generation of the inflammatory response, which occurs in patients with sepsis and ARDS.

The activity of protein kinases and consequent phosphorylation status of proteins is one of the main determinants of cellular enzyme activity and intracellular signaling mechanisms. Activity of protein kinase A, a cAMP-dependent kinase, has been shown to be increased in mechanically ventilated animals (54). Mitogen-activated protein kinases (MAPK) have been shown to be rapidly activated by mechanical strain in a human pulmonary epithelial cell line (12). At least three distinct families of MAPKs exist in mammalian cells: the p42/44 extracellular signal-regulated kinase (ERK) MAPKs, c-Jun NH₂-terminal kinases (JNK; JNK is also called stress-activated protein kinase [SAPK]), and p38 MAPK (12, 32). Mechanical stretch (5% strain 6 cycles/min for 2 h) activated SAPK in human lung epithelial cells (48). Moreover, high inspiratory pressure was shown to enhance phosphorylation of JNK and MAPK, which are not only inducers of gene transcription but may also be involved in the stretch-induced release of cytokines (48).

Recently, Bhattacharya et al. (7) found that lung expansion activated rat pulmonary endothelial tyrosine kinases may promote vascular remodeling in response to mechanical stresses. After induction with an inspiratory pressure of 22 cmH₂O, Western blots of cell lysates showed markedly enhanced phosphorylation of focal adhesion kinase, paxillin, and the tyrosine kinase Shc, which the authors speculated may function to stabilize endothelial cells to the cell matrix (7). In further studies, Parker et al. (46) used a phosphotyrosine phosphatase inhibitor (phenylarsine) and a tyrosine kinase inhibitor (genistein) to assess the role of these enzymes in pressure-induced lung injury. Inhibition of phosphotyrosine kinase was shown to lead to an increase in susceptibility of rat lungs to high positive inspiratory pressure (PIP) injury, whereas inhibition of tyrosine kinase attenuated the injury relative to the high PIP control lungs (46).

The signal transduction pathways that are currently thought to contribute to VILI are heterogeneous and, as of yet, poorly elucidated (see Fig. 1). What seems apparent is that the lung is a dynamic organ, subject to varying mechanical forces throughout life. The degree and magnitude of mechanical deformation observed with injurious forms of ventilation probably do not occur normally in nature. Therefore, it is unlikely that evolutionary mechanisms have been specifically developed to deal with the overdistension that can occur with mechanical ventilation. Consequently, the inflammatory response generated by mechanical ventilation might “borrow” signal transduction pathways from other more established signaling systems (76). Not surprisingly, the pathways favored are those that perceive the stimulus as noxious and respond by generating an inflammatory response.

Whether the inflammatory characteristics of VILI are simpler or more regulated than the ones seen in sepsis is at this point unclear. Notwithstanding this issue, although it has been demonstrated recently that lipopolysaccharide (LPS) incites its immune insult by interacting with one cellular receptor molecule, mechanical ventilation likely incites its inflammatory response through a combination of complex and possibly “redundant” mechanisms, more akin to the inflammatory response generated by microorganisms. The initial recognition of endotoxin by monocytes is dependent on the expression of CD14 and subsequent presentation of the endotoxin molecule to a member of the Toll receptor family (TLR-4), a highly evolutionary conserved family of transmembrane receptor proteins (73). The intracellular signaling through the TLR-4 receptor shows remarkable similarity to IL-1β signaling pathways. LPS has been shown to activate p42/44 MAPK, JNK, p38, p65, and NF-κB (73). Moreover, MacGillivray et al. (33) recently demonstrated that IL-1β receptor-associated kinase (IRAK) colocalizes with focal adhesion complexes and is required for IL-1β-dependent ERK activation (33). This suggests that the integrity of the actin filament arrays and the recruitment of IRAK into focal adhesion complexes are involved in the IL-1β-mediated signaling. It is unknown whether IL-1β-mediated cell signaling functions in a similar way in VILI.

As mentioned above, it has also been shown that mechanical forces activate the same signaling molecules in lung cells as LPS. Therefore, although it has not been demonstrated, it is postulated that signal transduction pathways specific to LPS might not only interact but also be common to those generated by VILI (26, 84). In fact, this observation might explain why VILI, endotoxin, and IL-1β administration to animals seem to cause similar effects (although evidence to the contrary is discussed below). Consequently, parallels between mechanisms of LPS-induced sepsis and VILI have been drawn. In addition, because Arbour et al. (3) demonstrated that individuals with cosegregating missense mutation affecting the extracellular domain of the TLR-4 receptor have a blunted response to inhaled LPS (3), the parallel between VILI and TLR-4/IL-1β signaling pathways has acquired novel and crucial clinical relevance. The current evidence raised the possibility that, at least in theory, the two pathways shared so much homology that mutations in the TLR-4/IL-1β receptor would not only confer resistance to sepsis but would also attenuate patient’s response to VILI.

Consequently, Uhlig et al. (72) examined the hypothesis that ventilation with large tidal volumes would result in similar responses as the lung stimulated with LPS. Using an isolated perfused lung model, they demonstrated activation of NF-κB in response to LPS and ventilation with large tidal volumes. This was abolished with treatment with dexamethasone (72). However, there were major differences in response to the two different stimuli when they used C3H/HeJ mice, which have abnormalities in the Toll receptor. As other
Fig. 1. Putative mechanisms of mechanotransduction in ventilator-induced lung injury (VILI). A: Ion channels. Mechanical stretch induces a Ca\(^{2+}\) influx via a mechanosensitive cation channel that has been shown to be inhibited by gadolinium. Mechanical stretch activates protein tyrosine kinases (PTK) and consequently activates phospholipase C-\(\gamma\) (PLC-\(\gamma\)) via its tyrosine phosphorylation. PLC-\(\gamma\) mediates the hydrolysis of phosphoinositol 4,5-bisphosphate (PIP\(_2\)) to generate inositol 1,4,5-trisphosphate (IP\(_3\)_ and diacylglycerol (DAG). IP\(_3\) mobilizes intracellular Ca\(^{2+}\). DAG in the presence of Ca\(^{2+}\) activates protein kinase C (PKC) and downstream events. PKC may activate transcriptional factors (c-fos) that bind to “stretch response elements” (SRE). Increased gene expression and production of cytokines and other pro- and anti-inflammatory molecules regulate the pathogenesis of VILI. B: Membrane disruption. Structural damage to cells causes elevations in intracellular free Ca\(^{2+}\) concentrations. Changes in Ca\(^{2+}\) homeostasis can affect signaling pathways and in sepsis has been shown to hypersensitize PKC activation. Traumatic breaks in the plasma membrane can induce activation of c-fos and translocation of nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) into the nucleus. PKC, activated NF-\(\kappa\)B, and c-fos can induce transcription of early response genes and bind to SRE to activate transcription. Increased gene expression and production of cytokines and other pro- and anti-inflammatory molecules regulate the pathogenesis of VILI. C: Cytoskeletal structure. The structural organization and interconnectedness of the cytoskeleton provides a physical basis for translating mechanical forces into biochemical responses. Mechanosensitive receptors and/or integrins are activated by mechanical forces. Integrins are known to maintain close relationships with focal adhesive kinase (FAK), kinases of the Src family, and paxillin. Through a series of as of yet unidentified pathways, members of the mitogen-activating protein kinases ([MAPK]; p38 and SAPK/JNK) and transcription factors (Erg-1) are activated. Subsequently, these proteins may activate NF-\(\kappa\)B and/or through other mechanisms gene transcription. Members of the integrin family can mediate NF-\(\kappa\)B regulation through activation of inhibitor of NF-\(\kappa\)B kinase (IKK). This in turn would mediate release of NF-\(\kappa\)B inhibition from inhibitor of NF-\(\kappa\)B (I\(\kappa\)B) by phosphorylation and subsequent translocation of NF-\(\kappa\)B to the nucleus. Dexamethasone can inhibit activation of NF-\(\kappa\)B. Activation of members of the MAPK family and NF-\(\kappa\)B can induce transcription of early response genes and bind to SRE to activate transcription. Increased gene expression and production of cytokines and other pro- and anti-inflammatory molecules regulate the pathogenesis of VILI. Color legend: Red: shown to occur in the lung and as a response to mechanical forces. Blue: demonstrated to occur in the lungs. Not known whether it occurs in response to mechanical forces consistent with VILI. Black: demonstrated to occur in cells. Not known whether it occurs in the lung in response to mechanical forces consistent with VILI. ECM, extracellular matrix; IC, intracellular.
investigators had shown previously, they found that there was no increase in TNF-α in response to LPS in these mice with no translocation of NF-κB. However, the mice ventilated with large tidal volumes had an increase in TNF-α of the perfusate and translocation of NF-κB (72). These studies suggest that there are fundamental differences between the upstream pathways generated by VILI vs. other inflammatory stimuli. Moreover, to some extent, they argue against the theory that mechanical stress “borrows” from inflammatory pathways and advocate for the reverse situation, in which pathways that evolved to respond to primordial stimuli, such as mechanical forces, were adapted by more advanced organisms to respond to more sophisticated signals such as inflammatory stimuli. Furthermore, the differing signal transduction pathways elicited by the septic stimulus (i.e., LPS) vs. the mechanical stimulus (i.e., VILI) opens up the possibility of targeted anti-inflammatory strategies for VILI, which would act on relatively specific pathways and hence may have fewer side effects with respect to infectious complications.

CELLULAR ACTIVATION AND CYTOKINE PRODUCTION

To understand the effects of the complex interplay between different inflammatory mediators in the organ system, it is essential to dissect the functional interactions involving the major cells known to play a role in the generation and propagation of VILI.

PMN leukocytes. PMN leukocytes have been unequivocally implicated in the pathogenesis of ALI and ARDS (15). The current literature also suggests that injurious ventilatory strategies used in normal lungs may increase cytokine production and neutrophil recruitment in lung lavage specimens (21). The current evidence seems to point to the role of PMNs as major effector cells in the generation of the tissue injury characteristic of VILI (66). One of the first studies proposing that mechanical ventilation could lead to an inflammatory response used a model of neutrophil depletion by nitrogen mustard. Kawano et al. (29) demonstrated that the neutrophil-depleted animals had markedly improved oxygenation and decreased pathological evidence of injury after lung lavage and/or mechanical ventilation vs. a control group treated with the lavage and ventilatory protocol alone.

More recently, Zhang et al. (83) combined these two observations and examined the hypothesis that mechanical ventilation could lead to activation of PMNS above and beyond that expected with ARDS alone. In these studies, PMNs isolated from normal human volunteers were incubated with BAL fluid from ARDS patients ventilated with either a conventional mechanical ventilation (CMV) or a protective ventilation strategy. Treated neutrophils were assessed postincubation for evidence of PMN activation as measured by oxygen burst index, expression of L-selectins, CD18 (ICAM-1), CD63, and elastase activity. This group (83) found that, in the conventional ventilation strategy group, all markers of neutrophil activation were increased more markedly than in the group ventilated with the protective strategy. These findings, although not conclusive, support the current evidence, which suggests that mechanical ventilation can lead to release of mediators that prime neutrophils, possibly providing a mechanism by which PMNs mediate tissue injury in VILI. Thus the role of PMNs in the pathogenesis of VILI may be regulated by their interaction with other cells, including epithelial cells and possibly vascular endothelial cells.

Endothelial cells. Pulmonary endothelial cells form a continuous monolayer on the luminal surface of the lung vasculature. Much is known about the response of vascular endothelial cells to shear stress (for a comprehensive review, see Refs. 13 and 52); however, relatively little is known about the response of pulmonary endothelial cells to mechanical stress generated by artificial ventilation. A major concept in vascular biology is that endothelial cells can become activated (85). Moreover, what has become evident is that only activated endothelium participates in the inflammatory response (15). In the human lung, endothelial cell stimulation with LPS, IL-1β, or TNF-α induces secretion of IL-8 and epithelial neutrophil-activating peptide (ENA-78), mediators of neutrophil sequestration and degranulation (6). Cyclic strain has also been shown to cause induction of immediate response genes such as the transcription factor activator protein-1 and NF-κB (16), the significance of which is unclear. Despite advances in endothelial biology, there is a paucity of data to explain the role of these cells in VILI. Currently, the search for the primary regulator of VILI has generated two important candidate cell types: alveolar macrophages and epithelial cells.

Alveolar macrophages. Over the past few years, alveolar macrophages have gained much attention as a source of cytokines, and it has been postulated that they function as important effector cells in the orchestration of VILI (17). Pugin et al. (47) reported that, in a plastic lung model, alveolar macrophages were responsible for the highest degree of response to mechanical stress as assessed by measuring IL-8 concentrations. Alveolar macrophages were also shown to play a role in lung remodeling, as they respond to mechanical stress by increasing de novo synthesis of matrix-metalloproteinase-9, a type IV collagenase (47). Monocyte-derived macrophages (MDM) produced responses that were similar to those of alveolar macrophages and were used in experiments as surrogate cells for alveolar macrophages. NF-κB was shown to undergo nuclear translocation in MDMs submitted to cyclic pressure stretching (47). Lentsch et al. (30) pointed to the essential role of alveolar macrophages in the activation of NF-κB. In alveolar macrophage-depleted rat lungs, the elevation of TNF-α and a CXC chemokine, macrophage inflammatory protein-2 (MIP-2), was suppressed and the upregulation of ICAM-1 was abrogated. Instillation of TNF-α restored NF-κB activation and inflammatory mediator responses (30). It has been inferred from these experiments that NF-κB translocation occurs in response to alveolar macrophage stretch and that this
may represent a fundamental contributing factor in the development of VILI; however, to date, this has not been conclusively demonstrated.

Alveolar epithelial cells. Another potential source of cytokines is alveolar epithelial cells, recently demonstrated to be a source of many cytokines and chemokines. Further studies from our laboratory (65), which used in situ hybridization and immunohistochemistry, suggest that the expression of TNF-α is significantly increased in the airway and alveolar epithelium after injurious ventilation. Our laboratory has recently demonstrated that primary cultured rat lung alveolar epithelial cells could produce TNF-α (35). Production of IL-8 has been found from a human carcinoma cell line (A549) (61). Vlahakis et al. (75) recently cultured these human alveolar epithelial cells on a deformable silicoelastis membrane. When stretched to 130% of baseline for 48 h, IL-8 secretion increased by up to 34% (75). Tsuda et al. (69) postulated that stretch of A549 cells alone did not significantly affect production of IL-8. Exploring a “synergism” hypothesis, they demonstrated that the physical stress exerted on the alveolar epithelial cells by a deposited asbestos fibers is greatly enhanced by cyclical stretch. Coating of fibers with fibronectin, a glycoprotein abundant in the alveolar lining fluid, significantly amplified the fiber-induced cell response. Furthermore, this response was inhibited by addition of an integrin-blocking agent to the culture medium, suggesting that adhesive interactions between protein-coated fibers and cell-surface molecules are involved in determining IL-8 secretion (69).

In an effort to further characterize the nature of the stretch response in epithelial cells, Mourgeon et al. (39) applied mechanical stretch to primary cultured fetal rat lung cells in an organotypic culture in which the three-dimensional culture system allowed fetal lung cells to form alveolar-like structures. With the use of this system, this group was able to demonstrate an additive effect between submaximal concentrations of LPS and mechanical stretch on production of MIP-2, a rodent homologue of human IL-8 (39) that can be produced by rat lung alveolar epithelial cells (81). LPS alone was associated with increases in MIP-2 mRNA levels, whereas stretch alone significantly increased MIP-2 production in the absence of de novo protein synthesis, implicating increased MIP-2 secretion as the possible effector mechanism of MIP-2 increase in epithelial cells activated by stretch (39). Because mechanical stretch is primarily applied to the alveolar septa, cytokines and chemokines produced from these cells could play an important role in mediating VILI.

THERAPEUTIC IMPLICATIONS

The realization that injurious ventilatory regimens are more deleterious when applied to injured or infected lungs has made a substantial impact on current thinking about ARDS and VILI. Three papers presented at the 2000 American Thoracic Society meeting in Toronto document this observation (2, 53, 55). This phenomenon, the “two hit hypothesis,” alludes to the fact that infectious/inflammatory insults act in a cumulative fashion. The initial stimulus generates an acute inflammatory reaction, akin to “priming,” that is apparently contained or restrained. The second stimulus, however, seems to mediate the loss of this compartmentalization or control and promotes the initiation of an inflammatory response that escapes regulatory mechanisms and may ultimately lead to MSOF and/or death.

Loss of compartmentalization has been discussed extensively in the context of sepsis, and it may be that it simply reflects the natural history of the inflammatory response gone awry (10). However, in the setting of mechanical ventilation, it is tempting to hypothesize that a particular factor(s) exists, which could potentially be amenable to extrinsic regulation, responsible for loss of inflammatory containment. Potential areas of future research include 1) irreversible loss of barrier function (epithelial and endothelial), 2) microorganism translocation and inflammatory amplification, 3) loss of surfactant anti-inflammatory properties, 4) impairment of alveolar/pulmonary repair mechanisms, 5) dysregulation of cellular mediators, 6) regulation of the anti-inflammatory response, and 7) loss of apoptotic control due to inflammatory changes. Although effector mechanisms likely work conjointly or synergistically in promoting SIRS and MSOF, the heterogeneity of the inflammatory response has become increasingly recognized. Further studies to characterize this response in different individuals will enable practitioners to tailor treatments to specific disease processes. For example, individuals with a propensity to develop high TNF-α levels due to polymorphisms in the TNF gene (36, 62) may benefit more (or less) from various treatment strategies. In view of recent evidence demonstrating that genetic polymorphisms may confer resistance to sepsis, understanding the genetic epidemiological “backdrop” in which inflammation/anti-inflammation occurs will likely be fundamental in determining treatment and predicting responses.

To date, much of the effort expended on immunotherapy, in the setting of sepsis and MSOF, has focused on anti-cytokine strategies. Interest in cytokine inhibition or blockade was stimulated by the finding that polyclonal antibodies to TNF-α prevented death during endotoxemia in mice (58). Unfortunately, results from human studies have not fulfilled their promise. The NORASEPT II study group recently published a double-blind randomized controlled trial of monoclonal antibody to human TNF-α in the treatment of 1,879 adult patients with septic shock (1). They were unable to demonstrate a significant difference between placebo and anti-TNF-α in 28-day mortality (1). Other studies of cytokine (i.e., IL-1) and chemokine (i.e., IL-8) inhibition have demonstrated positive results in animal models (58), but these results have not been transferable to humans. Notwithstanding the disappointing results of anti-cytokine therapy in patients with sepsis and MSOF, it must be noted that no study to our knowledge has looked specifically at the clinical effi-
cacy of anti-cytokine therapy in preventing MSOF in patients with ARDS.

Almost 60 randomized clinical trials have been undertaken that tested the hypothesis that modulation of the endogenous host inflammatory response can improve survival for patients with a clinical diagnosis of sepsis. In addition to increasing our comprehension about the intricate role of patient selection and improved appropriateness of outcome measures, the lessons learned for future trials in VILI include a realization that a single treatment modality is unlikely to significantly decrease mortality in this group of patients. Why should we think that anti-inflammatory therapies might be successful in the context of VILI, when they have not been successful in sepsis? There are a number of possible explanations for the lack of efficacy in sepsis trials (34, 82). One plausible explanation is that the clinical diagnosis of sepsis is made well after the initiation of the inflammatory stimuli; hence, therapy can only begin very late in the process. With VILI, we are in a unique position of knowing exactly when the inflammatory stimulus will begin (on initiation of mechanical ventilation) and hence could start anti-inflammatory therapy very soon after, or even before, the exciting stimulus begins.

Two papers have specifically addressed the issue of immunotherapy for VILI. Imai et al. (27) pretreated rabbits with both a high and low dose of polyclonal anti-TNF-α antibody. Animals subsequently underwent CMV. Pretreatment with intratracheally delivered polyclonal anti-TNF-α antibody improved oxygenation and respiratory compliance, decreased leukocyte infiltration, and ameliorated pathological findings. In another study, Narimanbekov and Rozyczki (43) used a recombinant IL-1 receptor antagonist before exposure to CMV. Pretreated rabbit BAL fluid showed lower albumin and elastase concentrations, as well as lower neutrophil count.

In addition to anti-cytokine therapies, the development of techniques for manipulating nucleic acids and strategies for delivering DNA to humans has made gene therapy a reality. Critical illness is probably a good target for gene therapy because of the high mortality and need for only transient treatments. To illustrate the potential applications of gene therapy in the management of ALI, Brigham and Stecenko (11) used a vector system that overexpressed the prostaglandin synthase gene in an in vivo model of ALI. This resulted in increased production of prostaglandin E₂ and prostacyclin in the in vivo lung and attenuation of the inflammatory response. Other promising therapeutic genes include genes coding for antioxidant enzymes, anti-proteases, or genes now shown to be specifically activated by mechanical stresses. Furthermore, on the basis of the information herein presented, antisense fos and overexpression of inhibitors of NF-κB and modulators of actin-myosin interaction may play an important role in the future, alone or as combination therapy, in the prevention of mechanical lung injury and consequently in the generation and propagation of the immune response underlying MSOF in ventilated patients with ARDS.

We thank Dr. M. Liu for careful review of this manuscript and helpful suggestions. This work was supported in part by the Medical Research Council of Canada Grant 8558.

REFERENCES


